OXFORD UNIVERSITY PRESS | Journal of Economic Entomology

Spatial distribution and sampling plans for Grape vine plant canopy inhabiting *Scaphoideus titanus* (Hemiptera, Cicadellidae) nymphs

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Rigamonti et al.: distribution and sampling plans for *S. titanus*

Journal of Economic Entomology
Sampling and Biostatistics

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4   inhabiting *Scaphoideus titanus* (Hemiptera, Cicadellidae) nymphs

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25 **ABSTRACT** The paper deals with the study of the spatial distribution and the design

26 of sampling plans for estimating nymph densities of the Grape leafhopper

27 *Scaphoideus titanus* Ball in vine plant canopies. In a reference vineyard, leaf

28 samples were repeatedly taken according to a multi-stage stratified random sampling

29 procedure and data were subjected to an ANOVA. There were no significant

30 differences in density neither among the strata within the vineyard nor between the

31 two strata with basal and apical leaves. The significant differences between densities

32 on trunk and productive shoots lead instead to the adoption of two-stage (leaves,

33 plants) and three-stage (leaves, shoots, plants) sampling plans for trunk shoots and

34 productive shoots inhabiting individuals respectively. The mean crowding to mean

35 relationship used to analyze the nymphs spatial distribution revealed aggregated

36 distributions. In both the enumerative and the sequential enumerative sampling

37 plans, the number of leaves of trunk shoots, and of leaves and shoots of productive

38 shoots, was kept constant while the number of plants varied. In additional vineyards

39 data were collected and used to test the applicability of the distribution model and the

40 sampling plans. The tests confirmed the applicability i) of the mean crowding to mean

41 regression model on the plant and leaf stages for representing trunk shoot inhabiting

42 distributions, and on the plant, shoot and leaf stages for productive shoot inhabiting

43 nymphs, ii) of the enumerative sampling plan, iii) of the sequential enumerative

44 sampling plan. In general, sequential enumerative sampling was more cost-efficient

45 than enumerative sampling.

46

47 **KEY WORDS** enumerative sampling plan, sequential enumerative sampling plan,

48 mean crowding to mean regression, reliability levels, simulation

49

50   The North American leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae)

51   arrived in Europe in the 1950s to reach quickly the status of key pest in European

52   vineyards (Bonfils and Schwester 1960, Schvester et al. 1961, 1962a, 1962b). It

53   invaded Southwestern and Southern European grapevine growing areas from where

54   it spread to Southeastern European regions. Currently, the area of distribution

55   spreads from Portugal to Bulgaria and is still expanding (Chuche and Thiery 2014).

56   *Scaphoideus titanus* is the vector of the *Candidatus* Phytoplasma vitis, a

57   Phytoplasma of the Elm Yellows or 16Sr-V group, an economically important A2

58   quarantine pest for EPPO causing the Flavescence dorée (FD). To plan and carry

59   out control operations, the occurrence of FD and its vector is monitored throughout

60   invaded and prospective areas of distribution (EPPO/CABI 1996).

61   Beating tray and sticky trap techniques are satisfactory for monitoring *S. titanus*

62   nymph and adult occurrences in the vine plant canopy and useful for validating short

63   and long term phenology models (Rigamonti et al. 2011, 2013, 2014) but inadequate

64   for obtaining intensities and absolute densities. Intensity estimates for vine plant leaf

65   inhabiting life stages obtained through visual examination of leaves proved to be

66   useful for between plant distribution studies, sampling plan design and canopy

67   infestation model development (Lessio and Alma 2006, Maggi et al. 2013). To obtain

68   absolute density estimates for nymphs, the proportion of nymphs inhabiting the

69   vegetation of the vineyard floor and the number of leaves per plant has to be taken

70   into account (Trivellone et al. 2011, 2013). Intensity estimates have long been used

71   for analysing spatial distributions in heterogeneous sampling universes (Southwood

72   1978). They improve the insight into population processes in spatially structured

73   environments and contribute to further developing supervised vector management

74   programs (Pedigo and Buntin 1994).

75   Already in the 1950s, LeRoux and Reimer (1959) used stratified multi-stage

76   sampling procedures in apple orchards. They randomly selected subsamples at

77   different stages that they stratified for taking into account stage-specific spatial

78   heterogeneities. The procedures have been detailed by Cochran (1977) and recently

79   applied to spatial studies of vineyard insects by Ifoulis and Savopoulou-Soultani

80   (2006). LeRoux and Reimer (1959) showed how to analyze the variance of within-

81   and between plant components and opened the door to the development of spatial

82   distribution models applicable to highly structured sampling universes, in that the

83   variance or related statistics are often regressed on mean intensity (Taylor 1961,

84   Iwao 1968, Kuno 1976). Since these models take into account the dependency of the

85   variance on the mean density, they are generally valid on a wide range of densities

86   (Davis, 1994).

87   For the estimation of the parameters of these models, quite large sets of data are

88   required. Since, for a given scale, the parameters tend to be species-specific and

89   constant through time and space, researchers rationalize data collection by applying

90   repeated sampling procedures (Iwao and Kuno 1971, Hagihara 1976, Taylor and

91   Woiwood 1980, Perry and Taylor 1986, Sawyer 1989).

92   The purpose of this paper is to apply multi-stage stratified random sampling

93   procedures to analyze the spatial distribution patterns of *S. titanus* in a highly

94   structured grape vine plant canopy of a FD free reference vineyard. Knowledge on

95   the sources of spatial variations can be used to set the stage for adapting Kuno's

96   (1976) regression model and employing it for modeling the spatial distribution and

97   designing sampling plans. A successful application of the distribution model and the

98   sampling plans in additional vineyards is expected to improve the sampling strategy

99   and to put adaptive *S. titanus* management in FD free areas on a more solid ground

100 than available so far.

101

Materials and Methods

103

104   **Study vineyards.** In a reference vineyard located at Camorino (46°09' N, 8°59' E,

105   220 m a.s.l.) in Southern Switzerland, the number of *S. titanus* individuals was

106   recorded repeatedly in 2009 to acquire data for analyzing the sources of variation,

107   distribution model development and sampling plan design.

108   Additional vineyards were sampled at Arbedo (46°13' N, 9°02' E, 240 m a.s.l.),

109   Bironico (46°07' N, 8°55' E, 490 m a.s.l.) and Contone (46°09' N, 8°56' E, 210 m

110   a.s.l.) to acquire data for testing the applicability of the distribution model and the

111   sampling plans

112   The plants were pruned to allow shoot development departing from 10 buds and

113   trained according to the Guyot double system. These shoots are referred to as

114   productive shoots that are different from the spontaneous unproductive trunk shoots.

115   Occasionally, the productive shoots were topped and hedged. Spontaneous

116   vegetation, occasionally mowed, covers the ground between the rows. Within the

117   rows, herbicides were used to control weeds. Synthetic pesticides were applied

118   against powdery and downy mildew but not against arthropods.

119   To facilitate the comparison with literature information, we simply refer to

120   'densities' rather than to 'relative densities' or 'intensities'. When referring to

121   subsampling, we refer to stages and levels in an interchangeable way.

122   **Sampling procedures.** LeRoux and Reimer's (1959) procedure takes into

123   account the spatial heterogeneity of the reference vineyard, the shoot type and the

124   within-shoot leaf position, and enhances the sampling efficiency through sub-

125   sampling the plant and the shoot. According to that procedure, to take into account

126   the possible within-vineyard heterogeneity, we stratified the reference vineyard to

127   obtain five plots of equal size, one at each side and one in the centre of the vineyard.

128   In each stratum, five plants were selected at random. Sub-sampling of the plant was

129   then carried out by randomly selecting three productive shoots and one trunk shoot.

130   To take into account a possible heterogeneity within productive and trunk shoots, all

131   shoots were divided into two equal strata, separating the leaves of the basal half from

132   those of the apical half. In each shoot stratum, three leaves were selected at random.

133   On each leaf, the number of *S. titanus* individuals was counted. The vineyard was

134   sampled weekly from 27 May 2009 to 30 July 2009. The samples taken on 27 May,

135   10 June, 17 June, 25 June and 3 July contained a sufficient number of nymphs for

136   conducting statistical analyses.

137   **Sources of variation.** To identify the sources of variation between sampling units

138   in the reference vineyard, analyses of variance (ANOVA) were carried out as

139   described by LeRoux and Reimer (1959). The between-plants variability is attributed

140   to the differences between the strata at the vineyard level and between the plants.

141   The within-plant variability is attributed to the differences between productive shoots

142   and the trunk shoots, and to the differences between the strata within the shoots. The

143   plant is considered as a random factor, as well as the productive shoots, while

144   vineyard strata, shoot type and shoot strata are considered as fixed. We followed

145   LeRoux and Reimer's (1959) recommendation of using untransformed counts.

146   **Spatial distribution model.** Iwao's (1968) model regresses Lloyd's (1967) mean

147   crowding index $\overset{*}{m}$ on mean density *m*, i.e.

148       $$\overset{*}{m} = \alpha + \beta\, m \qquad\qquad\qquad\qquad [1]$$

149   where *α* and *β* have been defined as index of basic contagion and density-

150   contagiousness coefficient, respectively, and qualified as meaningful indexes for the

151   spatial characteristics of a population (Davis 1994). Thereby, the parameters are

152　related to the basic population unit and to the aggregation of population units; the

153　basic unit is a colony, an individual or individuals repelling each other if $\alpha>0$, $\alpha=0$ or

154　$\alpha<0$, respectively, while the spatial distribution is aggregated, random or uniform if

155　$\beta>1$, $\beta=1$, $\beta<1$, respectively. Theoretical and experimental information suggests that

156　the regression parameters are species specific and constant through time and space,

157　for a given scale and range of densities (Iwao and Kuno 1971, Hagihara 1976, Taylor

158　and Woiwood 1980, Perry and Taylor 1986, Sawyer 1989). According to Kuno

159　(1976), the model holds for habitats of any given size, for a whole area as well as for

160　sub-samples.

161　　Kuno (1976) used model [1] to represent the distribution of insects in two- and

162　three stage random sampling programs. To facilitate the consultation of his paper, we

163　use whenever possible his symbols and terminology. Since the shoot types are the

164　most important source of variation (see the Results and Discussion section),

165　separate analyses for trunk shoot and productive shoot inhabiting populations are

166　carried out. The equation [1] then becomes

167　$m_j^* = \alpha + \beta_j\, m$ 　　　with *j=1,2* for trunk shoot distribution analyses 　　　[2]

168　$m_j^* = \alpha + \beta_j\, m$ 　　　with *j=1,2,3* for productive shoot distribution analyses 　　[3]

169　In the case of trunk shoots, the model is applied on the level of the vineyard (j=1),

170　with plants as primary sampling units (PSU), and on the level of the plant (j=2), with

171　leaves as secondary sampling units (SSU) (eq. [2]). In the case of productive shoots,

172　the model is applied on the level of the vineyard (j=1), with plants as primary

173　sampling units (PSU), on the level of the plant (j=2), with shoots as secondary

174　sampling units (SSU) and on the level of the shoot (j=3) with leaves as the tertiary

175　sampling unit (TSU) (eq. [3]).

176   Empirical information suggests that individuals are the basic unit of the population.

177   Waters *et al*. (2014) showed that modifying both the linear function and the statistical

178   model to force the intercept or lower functional limit through the origin results in more

179   intuitive biological interpretation of parameters and better sampling economy. Hence,

180   the regression models were forced through the origin at all stages under

181   consideration ($\alpha=0$). The mean crowding values were calculated for between plants,

182   between shoots and, for productive shoots only, within shoot relationships. The

183   parameters β_1 and β_2 for trunk shoot inhabiting individuals, and β_1, β_2 and β_3 for

184   productive shoots inhabiting individuals were obtained via least square regression

185   analyses.

186   **Reliability of density estimates and design of sampling plans.** The variance of

187   the density estimates for individuals living on trunk shoots and productive shoots can

188   be calculated with eqs. [2] and [6] in Kuno's (1976) paper. To introduce the

189   dependency of the variance to the mean, Kuno (1976) re-writes the variances in

190   terms of the relevant mean crowing $\overset{*}{m}$ to mean regression models (eqs. [2, 3]). For

191   assessing the reliability of ecological population estimates, the ratio D of the standard

192   error d to the mean m is used (Karandinos 1976, Kuno 1976, Pedigo and Buntin

193   1994).

194   The number of sampled units (leaves, shoots, plants) is small relative to the total

195   number of units, hence there is no need to make finite population corrections

196   (Cochran 1977), and Kuno's (1976) eqs. [16] and [25] can be used to develop

197   enumerative sampling plans.

198   For attaining a predefined reliability level D_0 with a fixed number k_0 of leaves per

199   plant, the number l of plants to be sampled, expressed as a function of the mean m

200   per leaf of individuals on trunk shoot leaves, is

201
$$l = \frac{\dfrac{1}{k_0}\left\{\dfrac{\alpha+1}{m} + \dfrac{\beta_1(\beta_2-1)}{\beta_2}\right\} + \dfrac{\beta_1-\beta_2}{\beta_2}}{D_0^{\,2} + \dfrac{1}{L}\dfrac{\beta_1-\beta_2}{\beta_2}}$$
  [4]

202 where α is the intercept, and β_1 and β_2 are the slopes of eq. [2], and in our study

203 $k_0=6$. For attaining a predefined reliability level D_0 with a fixed number k_0 of leaves

204 per shoot and a fixed number q_0 of shoots per plant, the number l of plants to be

205 sampled, expressed as a function of the mean m per leaf of individuals on productive

206 shoot leaves, is

207
$$l = \frac{\dfrac{1}{k_0}\left[\dfrac{\beta_1-\beta_3}{\beta_3} - \dfrac{\beta_1-\beta_2}{\beta_2} + \dfrac{1}{q_0}\left\{\dfrac{\alpha+1}{m} + \dfrac{\beta_1(\beta_3-1)}{\beta_3}\right\}\right] + \dfrac{\beta_1-\beta_2}{\beta_2}}{D_0^{\,2} + \dfrac{1}{L}\dfrac{\beta_1-\beta_2}{\beta_2}}$$
  [5]

208 where α is the intercept, and β_1, β_2 and β_3 are the slopes of eq. [3], and in our study

209 $k_0=6$ and $q_0=3$.

210 The high total number L of plants in the vineyard under consideration reduces $1/L$ to

211 zero and hence, the denominators to $D_0^{\,2}$. The computations are further facilitated

212 taking into account $\alpha=0$ as stated above.

213 The disregard of the finite population correction permits also the reference to

214 Kuno's (1976) eqs. [27] and [34] to develop sequential enumerative sampling plans.

215 According to this plan, the field work is terminated when the cumulative total count

216 T_n, with n being the sample size, *i.e.* the number of leaves sampled, reaches a

217 predetermined value.

218 The sequential estimation of the density $m=T_n/n$ per leaf of trunk shoot inhabiting

219 individuals is based on a constant number k_0 of leaves per plant. T_n is calculated after

220
$$T_n = \frac{\alpha+1}{D_0^{\,2} - \dfrac{1}{n}\left\{\dfrac{\beta_1(\beta_2-1)}{\beta_2} + \dfrac{\beta_1-\beta_2}{\beta_2}k_0\right\}}$$
  [6]

221   where $n = l\,k_0$, l being the number of plants, and $k_0 = 6$ in our study. For the sequential

222   estimation of the density $m = T_n/n$ of productive shoot inhabiting individuals, the

223   constancy of leaf numbers per shoot (k_0) and of shoot numbers per plant (q_0) leads to

224   calculate T_n as follows

225
$$T_n = \frac{\alpha + 1}{D_0{}^2 - \dfrac{1}{n}\left\{\dfrac{\beta_1(\beta_3 - 1)}{\beta_3} + \dfrac{\beta_1(\beta_2 - \beta_3)}{\beta_2\beta_3}q_0 + \dfrac{\beta_1 - \beta_2}{\beta_2}k_0 q_0\right\}} \qquad [7]$$

226   where $n = l\,q_0\,k_0$, l being the number of plants, $q_0 = 3$ and $k_0 = 6$. In eq. [6] and eq. [7], the

227   intercept α is set to 0.

Testing the applicability of the distribution model and the sampling plans

229   **Sampling procedures.** The sampling plans in eqs. [4, 5] were applied in the

230   Arbedo, Bironico and Contone vineyards where 100, 90 and 20 plants were selected

231   at random, respectively. The number of individuals was counted on six randomly

232   selected trunk shoot leaves, if the total number of leaves per trunk equaled or

233   exceeded 20. Trunks with a smaller number of leaves were disregarded and

234   substituted by another trunk. Among productive shoots, three were selected at

235   random on each plant, and nymphs were counted on six randomly selected leaves

236   per shoot. The Arbedo vineyard was sampled on 24 May 2011, the Contone vineyard

237   on 25 May 2011, and the Bironico vineyard on 12 June 2013 and on 1st July 2013.

238   The visits yielded four data sets that are used here for testing the applicability of the

239   spatial distribution model and the sampling plans.

240   **Applicability of the distribution model.** The sampling data obtained in the

241   additional vineyards were used to estimate the mean crowding to mean regression

242   statistics. Importantly, only a single sample was available in the Arbedo and Contone

243   vineyards, whereas the Bironico vineyard was sampled twice. Since the four samples

244   were treated separately, there were no replicates available for evaluating the slopes

245   at the plant stage. The samples were used to calculate via least square linear

246   regression analysis the parameters of eqs. [2, 3] with respective coefficient of

247   determination R^2 and standard errors. The comparison with the reference vineyard

248   statistics is made by evaluating the overlapping of confidence intervals at the

249   probability level $\alpha/2=0.05$ according to Payton et al. (2003).

250   **Applicability of the sampling plans.** To test the applicability of the enumerative

251   and sequential enumerative sampling plans, we calculated the precision levels on the

252   basis of the aforementioned four data sets, as detailed below. In the following the

253   index *c* was used to denote the precision levels (D_c) obtained.

254   To verify the applicability of the enumerative sampling plans, we computed

255   hypothesized densities based on unpublished monitoring data collected in previous

256   years in the additional vineyards by collaborators of the Agricultural Bureau of

257   Canton Ticino and of the Agroscope centre of excellence for agricultural research in

258   Switzerland. The resulting hypothesized densities were 0.36 and 0.26 individuals per

259   leaf on trunk shoots and on productive shoots, respectively. For these densities the

260   optimun sample size (OSS) for the $D_0=0.1$ and $D_0=0.3$ reliability levels, according to

261   eqs. [4, 5], were 77 and 9 plants for trunk shoots and 69 and 8 plants for productive

262   shoots respectively.

263   For each OSS, we carried out 1000 simulations with randomly selected plants in the

264   Arbedo, Bironico and Contone data sets, and estimated the densities. Based on

265   these densities and on the slopes β_l of the distribution model for the reference

266   vineyard, 1000 reliability values D_c were then computed, to finally yield average

267   values. The limited data set available for the Contone vineyard allowed the

268   estimation of means and reliability levels D_c for productive shoots at $D_0=0.3$ only. For

269   testing the enumerative sampling plan, the mean values D_c are compared with the

270   predefined $D_0=0.1$ and $D_0=0.3$ reliability levels.

271   To verify the applicability of the sequential enumerative sampling plans the four

272   data sets were used to simulate 1000 times the sequential sampling of nymphs on

273   trunk and productive shoots at the two levels of reliability. In each of the 1000

274   simulations, the number of plants required to meet or exceed the stop line for

275   estimates on trunk and productive shoots was obtained. As for the enumerative

276   sampling plan, based on the estimates of the density and on the slopes β_I of the

277   distribution model for the reference vineyard, 1000 reliability values D_c were then

278   computed, to finally yield average values. For testing the reliability of the sequential

279   sampling plan, the average D_c were compared with the predefined D_0=0.1 and D_0=0.3

280   reliability levels. The limited data set available from the Contone vineyard allowed the

281   estimation of means and reliability levels D_c for productive shoots at D_0=0.3 only.

282   For testing the efficiency of the sequential enumerative sampling plan in relation to

283   the enumerative sampling plan, the average number of plants examined for reaching

284   or exceeding the stop line was calculated and compared to the number used in

285   enumerative sampling plan.

286   **Statistical analysis.** All the statistical analysis were conducted using the IBM SPSS

287   Statistics 22 software (IBM Software Group 2014, Chicago, IL).

288

289   **Results and Discussion**

290

291   **Sources of variation in reference vineyard.** According to Table 1, there were no

292   significant differences between vineyard strata, indicating a homogeneous

293   environment between plants. However, there was a significant difference between

294   shoot types at the beginning of the growing season. This is explained by the behavior

295   of nymphs. Newly hatched nymphs mainly inhabit trunk shoot leaves, while older

296   nymphs disperse in the plant canopy, often after a passage through the vineyard

297   floor vegetation (Trivellone et al. 2011, 2013). Unexpectedly, there was no significant

298   difference within the shoot strata. Possibly, the topping of productive shoots induces

299   the growth of secondary shoots and creates a sampling environment in that the

300   stratification produced two subdivisions that differ in their distance from the trunk

301   rather than grouping leaves according to their age, indicating that shoots form a

302   homogeneous sampling environment.

303   Furthermore there were no significant differences between the grapevine plants.

304   This was unexpected because the within-plant variance is often smaller than the

305   between plant variance, allowing researchers to focus on plant samples and limit the

306   number of within-plant samples (Southwood 1978, Lessio and Alma 2006).

307   Nevertheless, the plants as primary units are retained in this and future works,

308   because the interactions between grape vines, FD and *S. titanus* presumably occur

309   on the plant level.

310   Based on the result of the ANOVA the stratification of the vineyard and the shoots

311   could be disregarded in regression model development and in the design of the

312   sampling plans. However, the difference between shoot types required a

313   differentiation between them and to work with two-stage sampling plans for trunk

314   shoot inhabiting individuals and with three-stage sampling plans for productive

315   shoots inhabiting individuals.

316   The mean densities per leaf reported in Table 1 are similar to the ones observed

317   by Lessio and Alma (2006) and by one of the authors of this paper (M. Jermini,

318   personal communication) in many untreated vineyards with comparable architectures

319   in Northwestern Italy and Southern Switzerland, respectively. Because of the

320   occurrence of similar densities, canopy structures and management procedures, the

321   Camorino reference vineyard is seen as representative for the many vineyards in an

322   extensive FD-free grape growing region and possibly beyond.

323   **Reference vineyard spatial distribution model.** Figures 1 and 2 depict the mean

324   crowding to mean relationship at the plant and leaf level for trunk shoot inhabiting *S.*

325   *titanus* and at the plant, shoot and leaf level for productive shoot inhabiting *S.*

326   *titanus,* respectively. By visual examination, Iwao's (1976) linear regression models,

327   with intercept α set to 0, appeared to describe satisfactorily the relationships. The

328   slopes β_1, β_2 and β_3 (in Fig. 2C only) were greater than 1 indicating an aggregated

329   distribution of population individuals as previously observed by Lessio and Alma

330   (2006). The aggregation on trunk shoot leaves was not unexpected since nymphs

331   may first aggregate in a highly heterogeneous vineyard floor to feed on preferred

332   plants and move to nearby vine plants thereafter (Trivellone et al. 2011, 2013).

333   **Reference vineyard sampling plans.** Figures 3 and 4 represent the enumerative

334   sampling plan for *S. titanus* individuals inhabiting trunk shoots and productive shoots,

335   respectively. The sampling plan referring to plants requires the taking of six leaves

336   per plant for trunk shoots or six leaves on each of three shoots for productive shoots.

337   The number of plants (*l*) depended on the density and the level of precision set to

338   D_0=0.1, adequate for research purposes, or D_0=0.3, suitable for pest management

339   activities.

340   This analysis deals with data obtained in vineyards in a FD free area. Presumably

341   the densities occurring in FD invaded areas are much smaller, leading to excessive

342   sample sizes for reliable population density estimates. For the time being this

343   possible disadvantage has no practical implications because in FD invaded areas the

344   mandatory treatments are undertaken without reference to population density

345   estimates.

346   Figures 5 and 6 represent the stop lines for the sequential enumerative sampling

347   for estimating the trunk shoot and productive shoot inhabiting individuals,

348   respectively. The optimum sampling plan referring to plants is designed for the

349   D_0=0.1 and D_0=0.3 reliability levels and requires the examination of six leaves per

350   plant or six leaves on each of three shoots for trunk shoot or productive shoots

351   inhabiting individuals, respectively.

352   The users of the sequential enumerative sampling plan have to control a minimum

353   number of plants, corresponding to the point at which the stop line reaches a positive

354   value, and, if required, continue to examine randomly selected additional plants until

355   the cumulative number of nymphs reaches the stop line. The minimum number of

356   plants depends on the shoot type and the level of precision D_0 and is 31 (for D_0=0.1)

357   or 4 (for D_0=0.3) when sampling trunk shoot inhabiting individuals, and 48 (for

358   D_0=0.1) or 6 (for D_0=0.3) when sampling productive shoot inhabiting individuals. In

359   both cases, the sequential method appears to be more efficient than the enumerative

360   plan since it often leads to a reliable estimate with a smaller number of examined

361   leaves.

362   **Spatial distribution in additional vineyards.** Table 2 shows the slopes with

363   associated standard errors of the mean crowding to mean regressions at the different

364   stages for trunk shoot and productive shoot inhabiting nymphs in the Bironico,

365   Contone and Arbedo vineyards. The slopes representing Iwao's (1968)

366   contagiousness show an aggregated distribution and moreover, little variability

367   among the samples. The highest degree of aggregation occurred on the shoot stage

368   for productive shoots, whereas the lowest degree of aggregation existed between

369   leaves on trunk shoots. The degree of aggregation was intermediate on leaves of

370   productive shoots.

371   The comparison of the average slopes given in Table 2 with means and

372   confidence intervals of the reference data set reported in Table 3 shows that all

373   means reported in Table 2 fall into the confidence interval obtained from Table 3.

374   Moreover, the confidence intervals given in Table 2 overlap with the confidence

375 intervals for the reference data set reported in Table 3. Hence, the parameter

376 estimates obtained from the Arbedo, Bironico and Contone can be considered in

377 agreement with the ones obtained in the Camorino reference vineyard.

378 Though the slope for densities on trunk shoots cannot be evaluated at the

379 vineyard stage (Table 2), it can be concluded that the spatial distribution model is

380 appropriate for the Arbedo, Bironico and Contone vineyards.

381 **Applicability of the sampling plans.** Table 4 shows that the number of plants,

382 calculated by applying the enumerative sampling plan to a hypothesized density,

383 yielded density estimates with mean reliability levels (columns 8 and 9) close to ones

384 predefined in the sampling plan (columns 4 and 5). This result was unexpected since

385 the hypothesized density (columns 2 and 3) used for determining the OSS was

386 generally different from the mean density obtained through simulations (column 6

387 and 7). Apparently, the enumerative sampling plans yielded density estimates at

388 satisfactory reliability levels even when using inaccurate hypothesized densities to

389 define the OSS. This is due to generally similar and relatively high densities observed

390 in the Arbedo, Bironico and Contone vineyards, a density range within that the OSS

391 changes little.

392 Table 5 shows that the average reliability levels D_c obtained through 1000

393 simulations are in general close to the predefined reliability levels D_0, from which we

394 can conclude that the sequential enumerative sampling plan with the statistics

395 obtained from the Camorino reference data set yielded reliable estimates for the

396 Arbedo, Bironico and Contone vineyards.

397 Table 5 also shows that, at least for the trunk shoot inhabiting nymphs, the number

398 of plants required to obtain a reliable density estimate was often but not always lower

399 than the number of plants used in the enumerative sampling plan. Hence, the

400 sequential enumerative sampling plan was in general more efficient than the

401 enumerative sampling plan. Table 5 also shows that the sequential enumerative

402 sampling plan is less efficient if the hypotesized density is much higher than the

403 estimated density.

404   The methodology presented shows how sampling procedures in a heterogeneous

405 sampling universe can be structured and prepared for spatial distribution modeling.

406 The study of the sources for the spatial variation within the canopy led to the design

407 of a multi-stage stratified random sampling program that could be simplified further

408 into a two- and a three stage random sampling program that did not require

409 stratification any more.

410   Nevertheless, a brief review of the modern literature suggests that the approach

411 outlined by Pottinger and Le Roux (1971), Kuno (1976), Cochran (1977) and

412 Southwood (1978) three decades ago did not receive sufficient attention in spatial

413 analyses and sampling plan design. Without studying the sources of spatial variation

414 in detail, the authors of papers on regression models rely on assumptions to proceed

415 to model development.

416   The mean crowding to mean regression model used at different stages in a highly

417 structured vine plant canopy appears to be appropriate to describe the distribution of

418 nymphs in the reference and additional vineyards. We presume that the model is also

419 applicable to additional vineyards with similar canopy architecture, spontaneous

420 vegetation and surroundings, but we see a need to evaluate the applicability in

421 vineyards with different characteristics. The slopes of the mean crowding to mean

422 regressions indicated an aggregated distribution on the plant, shoot and leaf levels.

423 The results obtained here may provide some support to the claims that the

424 regression parameters are constant through time and space, for a given scale and

425 range of densities (Iwao and Kuno 1971, Hagihara 1976, Taylor and Woiwood 1980,

426 Perry and Taylor 1986, Sawyer 1989), and the model holds for habitats of any given

427   size, for a whole area as well as for sub-samples (Kuno 1976). Undoubtedly, more

428   data should be analyzed to confirm the validity of this claim.

429   Moreover, the results show that both the enumerative and the sequential

430   enumerative sampling plans can be applied to other vineyards with similar canopy

431   architecture, spontaneous vegetation and surroundings. To be able to apply

432   efficiently the enumerative sampling plan, the user should have some information on

433   possible densities in the vineyard to be sampled. This information doesn't have to be

434   accurate, however, and could be acquired in a preliminary sampling program or, as

435   done here, by approximating densities on the basis of previously conducted

436   monitoring programs. The results show that satisfactory levels of reliability are

437   reached even if the density estimate for determining the OSS is considerably

438   different from the actual density. This result may be of interest to pest managers

439   favoring enumerative over sequential enumerative sampling plans. As stated in the

440   case of the distribution model, the results obtained here should be critically evaluated

441   for other populations in different environments before drawing general conclusions.

442   The number of samples to be taken in the enumerative sampling program is higher

443   than the number reported by Lessio and Alma (2006). To some extent, the difference

444   may be due to different reliability levels for density estimates and the use of different

445   regression models that both can be justified (Davis 1994). Indeed, in contrast to this

446   work, Lessio and Alma (2006) relied on an application of Taylor's (1961) power law.

447   To a greater extent, however, the difference is due to the spatial variation under

448   consideration. Whereas Lessio and Alma (2006) neglected within-plant sources of

449   variability and focused on between-plant variation, the present findings show that the

450   within plant variability is more important than the between-plant variability and should

451   consequently be taken into account.

452   The sequential enumerative sampling plan can be applied to other vineyards with

453   a similar canopy architecture, spontaneous vegetation and surroundings. As

454   expected the sequential enumerative sampling plan is in general, but not always,

455   more cost efficient than the enumerative sampling plan. In sequential enumerative

456   density estimation, the applicability of the sampling plan doesn't require previous

457   knowledge on infestation levels and the effort to estimate population density

458   promises to be smaller than in the case of enumerative sampling. If, however, the

459   accumulated number of samples exceeds the one found in the enumerative sampling

460   plan, the user is advised to terminate the sampling activities and rely on the reliability

461   of the enumerative sampling plan.

462   The successful application and the applicability of the sampling plans in additional

463   vineyards put adaptive *S. titanus* management on more solid grounds than available

464   so far. So far, the *S. titanus* adaptive management project emphasized the within

465   vineyard population dynamics and the development of respective adaptive

466   management strategies. For this purpose the enumerative and sequential

467   enumerative sampling plans presented here are particularly appropriate and a

468   reliability level of D_0=0.1 was considered adequate. In the context of regional

469   population studies and disease transmission, however, the pest manager may be

470   interested in regional sampling and management operations. For this purpose, we

471   tentatively proposed a D_0=0.3 reliability level. As shown already by Sawyer and

472   Haynes (1985), regional population estimations require the extension of the spatial

473   model and the possible consideration of additional strata at the landscape level.

474   Further improvements of the sampling strategy for adaptive *S. titanus*

475   management can be made when re-orienting the work from research to adaptive

476   management under specific agricultural conditions. For example, improvements are

477   possible when focusing on initial densities on trunk shoots to decide on the necessity

478   of including the vineyard in a monitoring program. If a threshold density for this

479   decision can be given and the nymph density is at moderate levels, cost efficient

480   presence-absence sampling plans with respect to a threshold can be developed and

481   implemented (Wilson *et al.*, 1983; Bianchi *et al.*, 1989, Knapp *et al.*, 2006). Further

482   improvements are possible when focusing on specific developmental stages whose

483   occurrence may trigger a chemical control operation (*e.g.* in Switzerland the

484   appearance of third instar nymphs is used to determine the timing of the first

485   mandatory treatment). Guided by the results of continuously adapted phenology

486   simulations, the pest manager should be able to reduce the frequency of sampling

487   operations (Jermini *et al.*, 2013; Prevostini *et al*, 2013).

488   The model describes the intensity per leaf of nymphs only. The analysis of the

489   spatial distribution of bark inhabiting eggs and mobile adults requires a different

490   sampling technique and a revision of the model structure. To obtain absolute density

491   estimates for all life stages from intensity estimates, the proportion of vineyard floor

492   inhabiting nymphs, which depends from the botanical composition, and the number

493   of leaves per plant has to be taken into account (Trivellone *et al.*, 2013).

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495   **Acknowledgments**

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617   **Table 1. Analysis of variance for visual *S. titanus* counts made on five sampling**

618   **dates in a reference vineyard at Camorino (Switzerland) (DF = degrees of freedom, MS**

619   **= observed mean squares, F = F-value; vineyard strata, shoot type and shoot strata**

620   **are considered as fixed factors, while plants are considered as random factors; **:**

621   **significant at *P*<0.01)**

Sampling date (mean density)		May 27 (0.954)		June 10 (0.790)		June 17 (0.536)		June 25 (0.280)		July 3 (0.170)	
Source of variability	DF	MS	F	MS	F	MS	F	MS	F	MS	F
Between plants											
Vineyard strata	4	18.48	1.44	14.79	1.92	1.72	0.94	0.65	0.77	1.02	1.21
Plants	20	12.78	1.04	7.70	0.89	1.83	0.70	0.84	1.32	0.84	1.41
Within plants											
Shoot type	1	82.23	6.69**	178.20	20.64**	15.74	6.03**	0.04	0.06	1.62	2.07
Shoot strata	1	23.48	1.91	4.61	0.53	3.13	1.20	0.13	0.21	1.46	2.44
Residual	73	12.30		8.63		2.61		0.64		0.60	

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626   **Table 2. Mean crowding to mean regression statistics for *S. titanus* nymphs**

627   **inhabiting trunk and productive shoots in the in the Arbedo, Bironico and Contone**

628   **vineyards (for testing overlapping distributions, the confidence interval at the $\alpha/2=0.05$**

629   **probability level is calculated after Payton et al. (2003)).**

Sample	Slope and confidence interval for trunk shoot inhabiting individuals	Slope and confidence interval for productive shoot inhabiting individuals	
	Stage 1	Stage 2	Stage 1
	Between leaf relationship	Between shoot relationship	Between leaf relationship
Arbedo vineyard	1.35 ± 0.1153	2.75 ± 0.4453	2.05 ± 0.1797
Bironico vineyard, 12June 2013	1.49 ± 0.1319	2.31 ± 0.3133	1.79 ± 0.1471
Bironico vineyard, 1st July 2013	1.36 ± 0.2144	2.34 ± 0.3958	1.95 ± 0.19661
Contone vineyard	1.28 ± 0.0208	2.42 ± 0.7099	1.67 ± 0.2988

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634   **Table 3. Mean crowding to mean regression statistics for *S. titanus* nymphs**

635   **inhabiting trunk and productive shoots in the Camorino reference vineyard (for testing**

636   **overlapping distributions, the confidence interval at the α/2=0.05 probability level is**

637   **calculated after Payton et al. (2003)).**

Stage	Slope	Standard error (SE)	R^2	n	Confidence interval
		Trunk shoots			
Plant (β_1)	1.6784	0.1735	0.9045	5	0.3702
Leaf (β_2)	1.3603	0.7895	0.6344	79	1.3040
		Productive shoots			
Plant (β_1)	2.8444	0.167	0.9563	5	0.3564
Shoot (β_2)	2.1325	0.960	0.6766	123	1.5777
Leaf (β_3)	1.8686	0.490	0.7398	296	0.8012

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642   **Table 4. The applicability of the enumerative sampling plan: the hypothesized mean**

643   **densities with the required number of plants to be sampled, and means and reliability**

644   **levels (mean values) obtained from 1000 simulated sampling procedures vineyards**

645   **(nc = sample size too small for the calculations; the predefined reliability level D_0 is**

646   **reported in parentheses).**

Vineyard	Hypothesized density on		Number of plants selected for		Estimated mean (1000 simulations) on		Reliability D_c (1000 simulations), mean values, on	
	trunk shoots	productive shoots	trunk shoot estimates	productive shoot estimates	trunk shoots	productive shoots	trunk shoots	productive shoots
Arbedo	0.36	0.26	77 (0.1)	69 (0.1)	0.96	0.22	0.09	0.11
			9 (0.3)	8 (0.3)	0.96	0.22	0.25	0.32
Bironico 12 June 2013	0.36	0.26	77 (0.1)	69 (0.1)	0.83	0.26	0.10	0.12
			9 (0.3)	8 (0.3)	0.83	0.26	0.30	0.34
Bironico 1st July 2013[t]	0.36	0.26	77 (0.1)	69 (0.1)	0.17	0.18	0.16	0.10
			9 (0.3)	8 (0.3)	0.17	0.18	0.43	0.28
Contone	0.36	0.26	nc (0.1)	nc (0.1)	nc	nc	nc	nc
			9 (0.3)	8 (0.3)	nc	0.18	nc	0.33

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650 **Table 5. The applicability of the sequential enumerative sampling plan: the most**
651 **frequent number of plants required to be sampled for reaching or exceeding the stop**
652 **line and the reliability levels (mean values) obtained from 1000 simulated sampling**
653 **procedures (nc = sample size too small for the calculations).**

Vineyard	Shoot type	Most frequent number of plants required to meet the stop line	Calculated reliability (D_c), mean values
Predefined reliability $D_0 = 0.1$			
Arbedo	Trunk shoots	67	0.096
	Productive shoots	73	0.102
Bironico	Trunk shoots	69	0.105
12 June 2013	Productive shoots	70	0.117
Bironico	Trunk shoots	149	0.113
1st July 2013	Productive shoots	78	0.087
Contone	Trunk shoots	nc	nc
	Productive shoots	nc	nc
Predefined reliability $D_0 = 0.3$			
Arbedo	Trunk shoots	8	0.246
	Productive shoots	9	0.284
Bironico	Trunk shoots	8	0.296
12 June 2013	Productive shoots	9	0.308
Bironico	Trunk shoots	18	0.305
1st July 2013	Productive shoots	10	0.248
Contone	Trunk shoots	nc	nc
	Productive shoots	11	0.316

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657   **Fig. 1.** Mean crowding ($\overset{*}{m}$) to mean (m) regression statistics (density-

658   contagiousness coefficient β with SE = standard error, and coefficient of

659   determination R^2) for *S. titanus* nymphs inhabiting trunk shoots of grapevine plants in

660   the Camorino reference vineyard (A and B refer to the plant and leaf level,

661   respectively).

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664   **Fig. 2.** Mean crowding ($\overset{*}{m}$) to mean (m) regression statistics (density-

665   contagiousness coefficient β with SE = standard error, and coefficient of

666   determination R^2) for *S. titanus* nymphs inhabiting productive shoots of grapevine

667   plants in the Camorino reference vineyard (A, B and C refer to the plant, shoot and

668   leaf level, respectively).

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671   **Fig. 3.** Enumerative sampling plan with optimal sample size for *S. titanus* nymphs

672   inhabiting trunk shoot leaves. The solid and dotted lines represent the number of

673   plants to be sampled in relation to *S. titanus* density, at precision levels D_0=0.1 and

674   D_0=0.3 respectively.

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677   **Fig. 4.** Enumerative sampling plan with optimal sample size for *S. titanus* nymphs

678   inhabiting productive shoot leaves. The solid and dotted lines represent the number

679   of plants to be sampled in relation to *S. titanus* density, at precision levels D_0=0.1 and

680   D_0=0.3 respectively.

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683   **Fig. 5.** Sequential enumerative sampling plan for *S. titanus* nymphs inhabiting

684   trunk shoots (A: stop line for D_0=0.1; B: stop line for D_0=0.3).

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687   **Fig. 6.** Sequential enumerative sampling plan for *S. titanus* nymphs inhabiting

688   productive shoots (A: stop line for D_0=0.1; B: stop line for D_0=0.3).
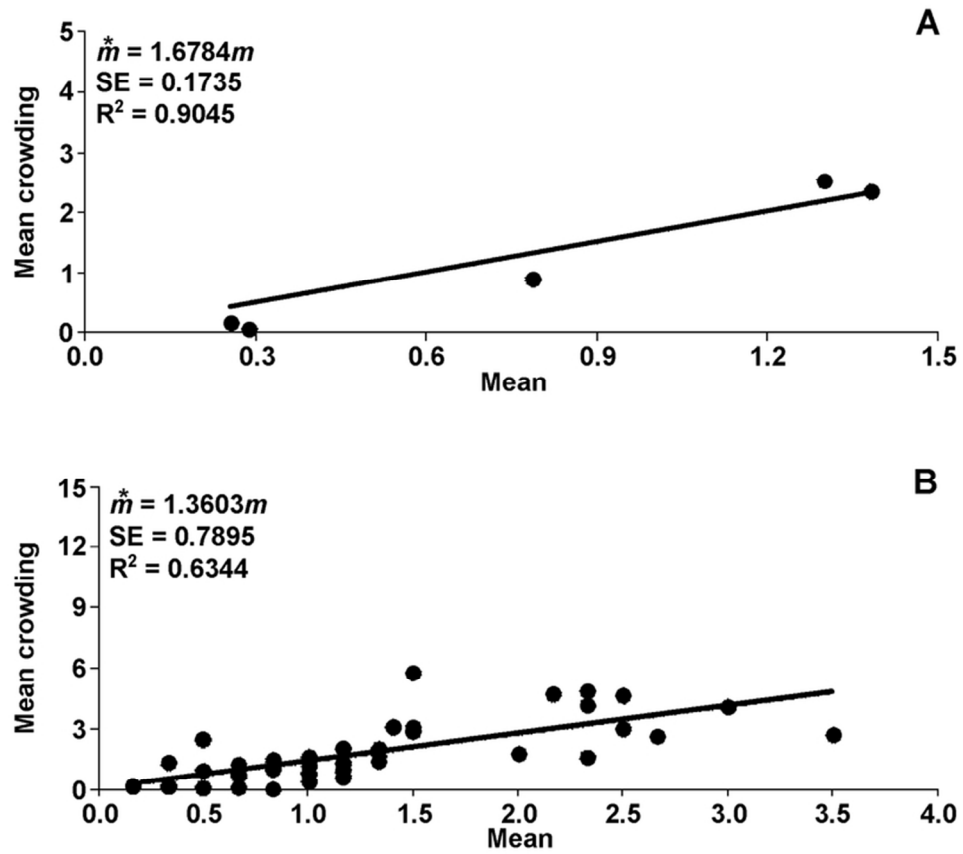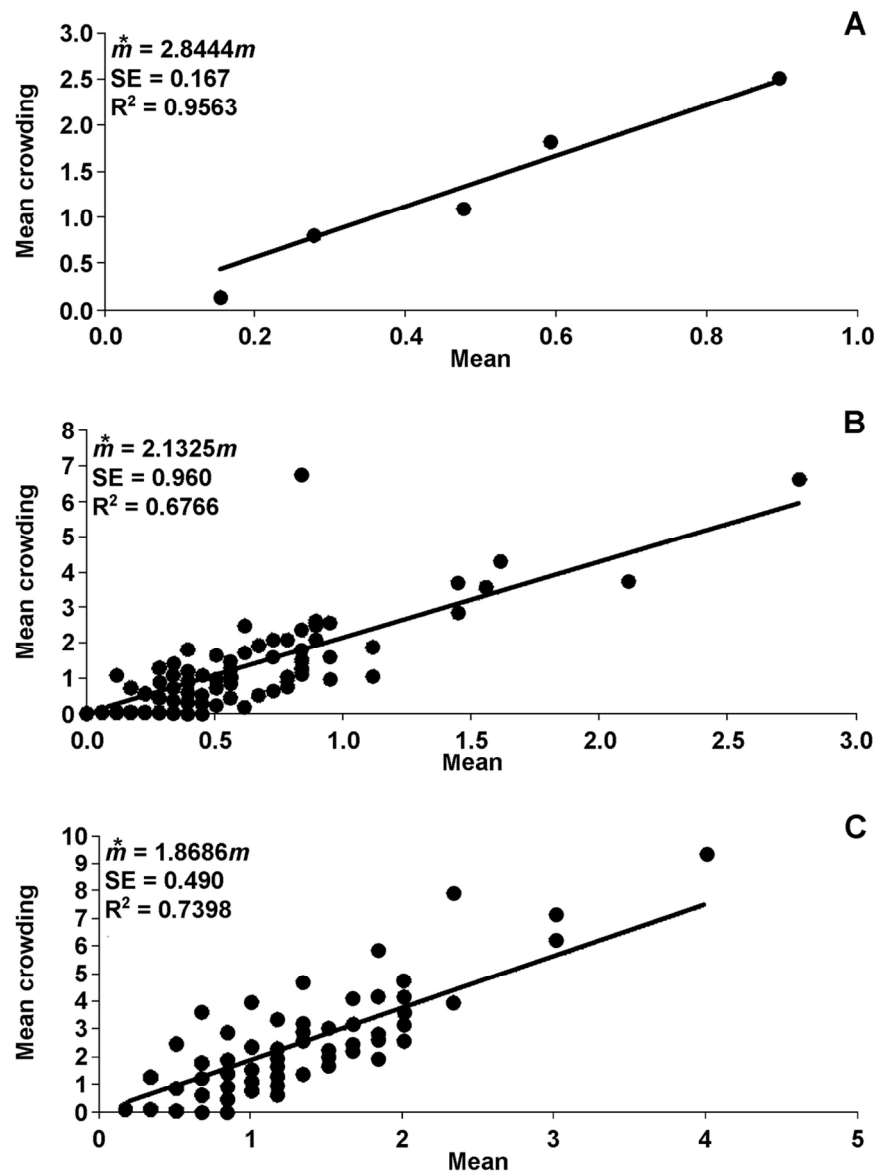
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Fig. 1. Mean crowding (m^*) to mean (m) regression statistics (density-contagiousness coefficient ββwith SE = standard error, and coefficient of determination R^2) for *S. titanus* nymphs inhabiting trunk shoots of grapevine plants in the Camorino reference vineyard (A and B refer to the plant and leaf level, respectively). 71x64mm (300 x 300 DPI)

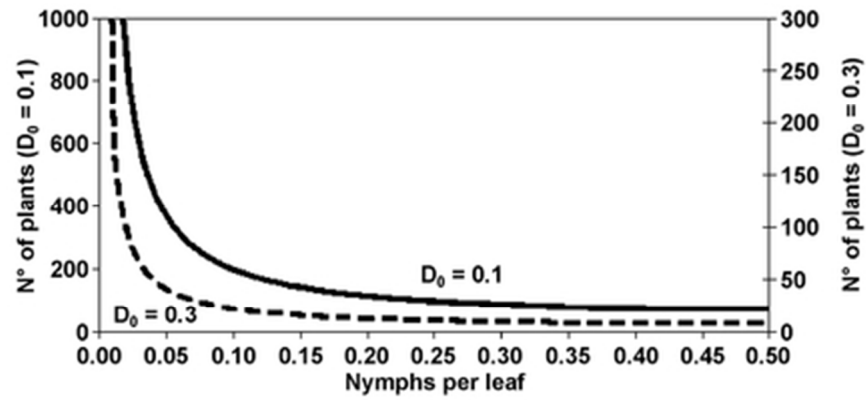Fig. 2. Mean crowding (m^*) to mean (m) regression statistics (density-contagiousness coefficient β with SE = standard error, and coefficient of determination R^2) for *S. titanus* nymphs inhabiting productive shoots of grapevine plants in the Camorino reference vineyard (A, B and C refer to the plant, shoot and leaf level, respectively).
105x138mm (300 x 300 DPI)

Fig. 3. Enumerative sampling plan with optimal sample size for *S. titanus* nymphs inhabiting trunk shoot leaves. The solid and dotted lines represent the number of plants to be sampled in relation to *S. titanus* density, at precision levels $D_0=0.1$ and $D_0=0.3$ respectively.
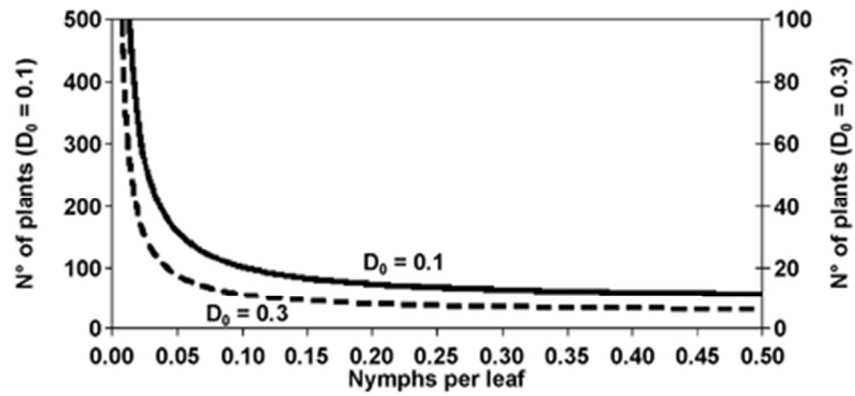36x17mm (300 x 300 DPI)

Fig. 4. Enumerative sampling plan with optimal sample size for *S. titanus* nymphs inhabiting productive shoot leaves. The solid and dotted lines represent the number of plants to be sampled in relation to *S. titanus* density, at precision levels $D_0=0.1$ and $D_0=0.3$ respectively.
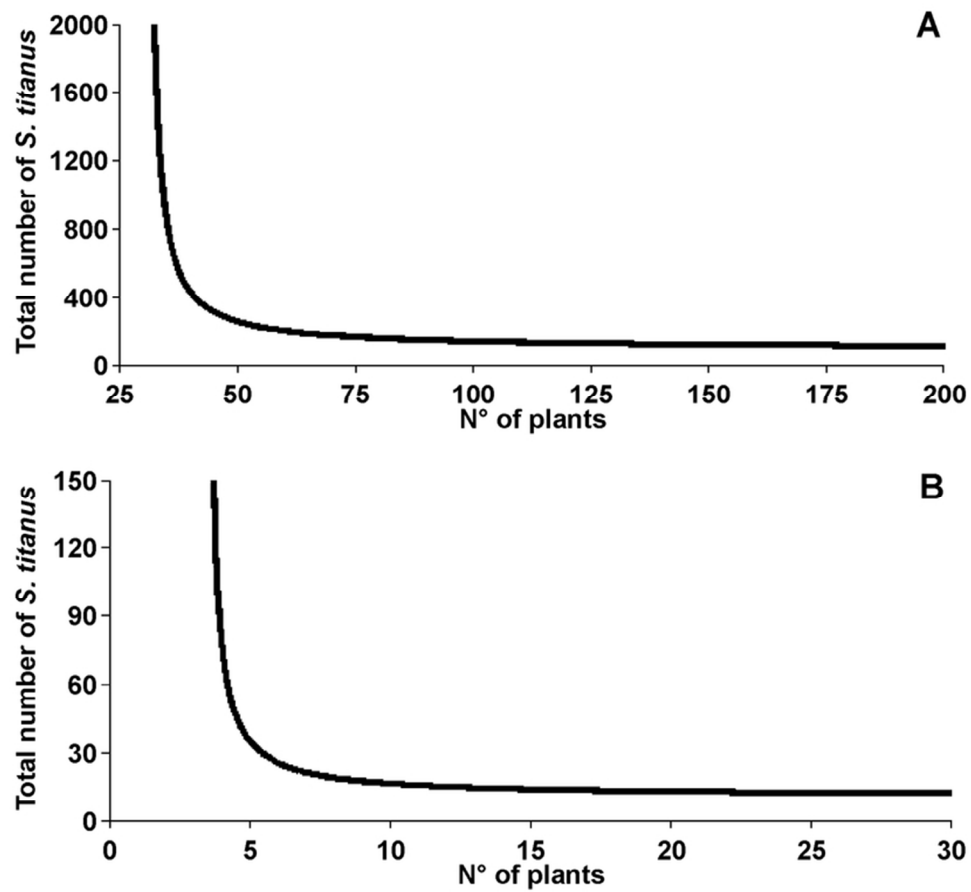36x16mm (300 x 300 DPI)

Fig. 5. Sequential enumerative sampling plan for *S. titanus* nymphs inhabiting trunk shoots (A: stop line for $D_0=0.1$; B: stop line for $D_0=0.3$).
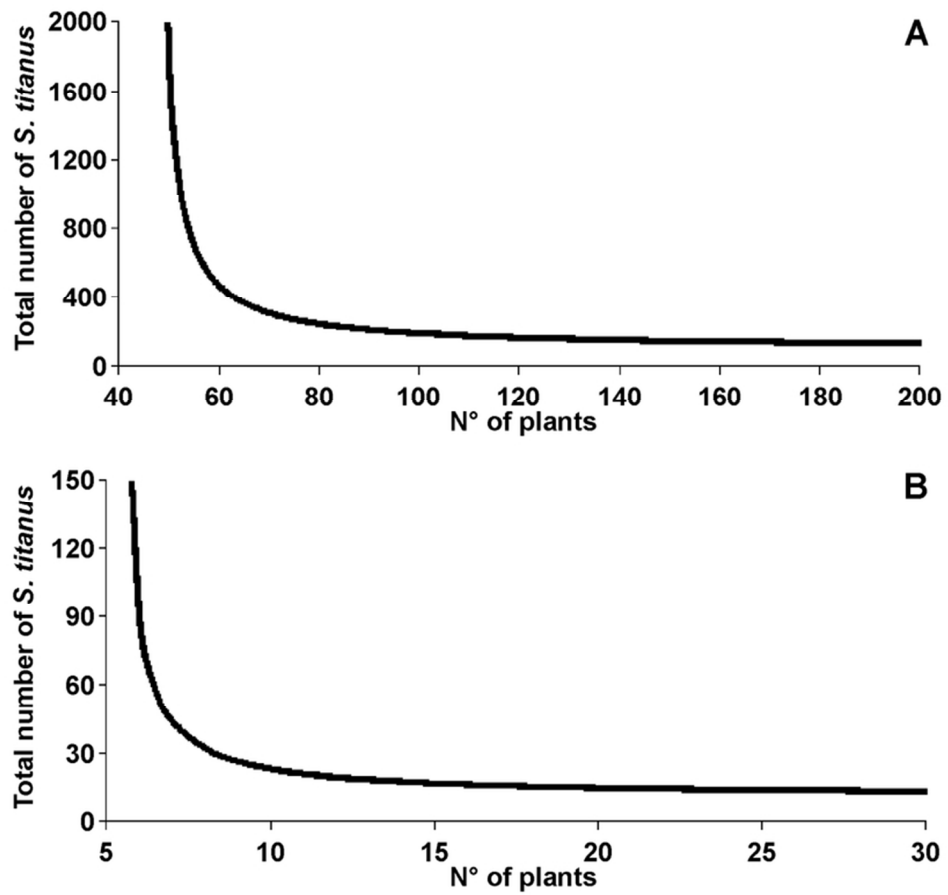72x65mm (300 x 300 DPI)

Fig. 6. Sequential enumerative sampling plan for *S. titanus* nymphs inhabiting productive shoots (A: stop line for $D_0=0.1$; B: stop line for $D_0=0.3$).
73x67mm (300 x 300 DPI)